The role of regulatory variation in complex traits and disease

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Abstract | We are in a phase of unprecedented progress in identifying genetic loci that cause variation in traits ranging from growth and fitness in simple organisms to disease in humans. However, a mechanistic understanding of how these loci influence traits is lacking for the majority of loci. Studies of the genetics of gene expression have emerged as a key tool for linking DNA sequence variation to phenotypes. Here, we review recent insights into the molecular nature of regulatory variants and describe their influence on the transcriptome and the proteome. We discuss conceptual advances from studies in model organisms and present examples of complete chains of causality that link individual polymorphisms to changes in gene expression, which in turn result in physiological changes and, ultimately, disease risk.

Expression quantitative trait loci
(eQTLs). Genomic regions that carry one or more DNA sequence variants that influence the expression level (typically mRNA abundance) of a given gene.

Recombinant offspring
Offspring of sexually reproducing organisms that carry a random combination of the alleles that they have inherited from their parents.

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doi:10.1038/nrg3891
Published online 24 February 2015

Propelled by technological advances, we will soon have essentially complete catalogues of all but the rarest genetic variants in humans and several other species. Individuals in most species differ from each other by thousands to millions of DNA sequence variants. Some of this variation contributes to observable phenotypic differences in traits ranging from morphology, physiology and behaviour to predisposition to many human diseases. While the identification of variants that affect phenotypes is rapidly progressing, the fundamental challenge now is to understand how these variants exert their effects.

An important class of variants, termed expression quantitative trait loci (eQTLs), influence the expression level of genes (BOX 1). The genetics of expression variation of single genes has been studied since at least 1962 (REF. 1). Genome-wide eQTL mapping was proposed in 2001 (REF. 2) and first carried out in its modern form at about the same time in a cross between two yeast strains[3]. Brem *et al.*[3] used microarrays to measure variation in mRNA abundance for all expressed genes among recombinant offspring of these two parent strains. Relating this variation to the alleles that each offspring inherited from either parent allowed the identification of regions in the genome that harbour sequence variants that influence gene expression. The now-ubiquitous term eQTL for such regions was coined shortly thereafter[4], although the related term protein quantity loci (pQLs; now more commonly known as protein QTLs (pQTLs)) was used in an examination of the genetics of protein levels for a limited number of genes in 1994 (REF. 5). Since then, there has been tremendous progress in the study of regulatory variation. Maps of eQTLs are being built in increasingly large-scale studies in humans[6–12] (see REF. 13 for earlier landmark studies) (TABLE 1), rodents[4,14–20], flies[21,22], plants[4,23–30], worms[31,32] and other species. The early observations in yeast of local and distant eQTLs, eQTL hot spots, a complex genetic basis of expression traits, and connections between expression and organismal phenotypes[3,33] (see below) have since been found to hold in other species.

Beyond ever larger catalogues of eQTLs, our understanding is now being expanded in two directions. Although eQTLs were typically identified as 'loci' — that is, statistical associations between regions of the genome and the expression of genes — the identity of the precise causal variants and their molecular mode of action are coming into increasingly sharper view. Additionally, there is a growing understanding of the consequences of variation in gene expression levels for organisms. This second aspect is especially important because a crucial rationale for large eQTL studies is that they can help to prioritize likely causal variants among the multiple polymorphisms within the regions identified by genome-wide association studies (GWASs), as well as to reveal the precise biological mechanisms through which DNA differences influence organismal traits. For example, the majority of loci identified in human GWASs are found in non-coding regions that are not in linkage disequilibrium with coding exons and must therefore reflect the effects of regulatory variation[34].

Protein QTLs
(pQTLs). Genomic regions that carry one or more DNA sequence variants that influence the protein abundance of a given gene.

eQTL hot spots
Regions of the genome that contain more expression quantitative trait loci (eQTLs) than expected by chance.

Previous reviews have covered the various types of eQTLs and the ways in which they can be identified and fine-mapped[13,35–37], the rich variety of molecular traits that can be assayed along the cascade of gene expression regulation[38,39] and the ways to integrate these molecular traits in a systems genetics perspective[40]. Here, we review new insights into the molecular basis of eQTLs and the genetics of mRNA versus protein levels. We then present recent discoveries into the causal links between eQTLs and higher-order organismal phenotypes, such as physiology and disease. We describe recent experimental insights into eQTL causality (many of which were derived from model organisms) and close by presenting an overview of the emerging evidence for eQTL causality in human disease.

What are eQTLs?

eQTLs contain sequence variants that affect the expression of a gene. They are similar to other QTLs that can influence any given trait of interest (for example, height, growth rate and disease risk) except that the trait under study is gene expression. eQTLs are identified by measuring gene expression in panels of genetically different, genotyped individuals[13,36] (BOXES 1,2). These panels can be

Box 1 | **A beginner's guide to eQTL mapping**

Expression quantitative trait loci (eQTLs) are regions of the genome containing DNA sequence variants that influence the expression level of one or more genes. They are identified by studying a population of genetically different individuals (FIG. 1). These individuals can be members of an outbred population (for example, human individuals) or can be bred using experimental crosses (for example, from a cross between two genetically different yeast strains or a panel of mouse strains). The individuals in the population differ from each other at many sequence variants, from tens of thousands in yeast crosses to millions in human populations. Most of these variants do not have any consequences on gene expression (or on any other trait).

To identify the comparatively few variants that influence gene expression, two types of data are collected from each individual. First, each individual needs to be genotyped. If the sequence variants in the population are known, genotyping can be done by targeted assays of each variant in each individual (for example, using single-nucleotide polymorphism (SNP) microarrays). Otherwise, current technologies now allow the genome of each individual to be fully sequenced so that all variants are discovered. Second, the expression of each gene in the genome is measured in each individual using either expression microarrays or RNA sequencing. eQTLs are then identified by comparing the genotypes with the expression levels using association (in outbred populations) or linkage analysis (in pedigrees or designed crosses).

To test whether a given sequence variant has an effect on the expression of a given gene, a statistical test is performed (see the figure, part **a**). Individuals are grouped according to the allele they carry. If the gene has a significantly higher expression level in one group than in the other group, we can conclude that the variant (or another variant in linkage disequilibrium) influences the expression of this gene. The test is repeated at every DNA variant in the genome, resulting in a genome scan for eQTLs for this gene (see the figure, part **a**).

The figure (part **a**) shows a genome scan for mRNA levels of the yeast *TPO1* gene in a cross between two yeast strains. The logarithm of the odds (LOD) score is a measure of the strength of the statistical association between mRNA level and genotype. Light blue shapes show the distribution of expression levels, and blue dots are expression levels for individual segregants. The thick black bars show the central 50% of the data, and the white dot indicates the median. When mRNA levels are significantly higher in individuals that have inherited one allele than those that have inherited the other allele, the LOD score is high and the region is called an eQTL. An example is shown on the left end of chromosome 15 where the LOD score exceeds the genome-wide threshold (indicated by the dashed red line). When there is no difference in mRNA levels between genotype groups, the LOD score is low (see the example region on chromosome 4). The genome scan is repeated for the expression of every gene in the genome (see the figure, part **b**). Shown here are the LOD profiles for 200 randomly selected genes. The genes are sorted according to their genomic position. Local eQTLs form a diagonal, and eQTL hot spots are visible as vertical (for example, on chromosomes 14 and 15).

The figure was generated using data from REF. 50.

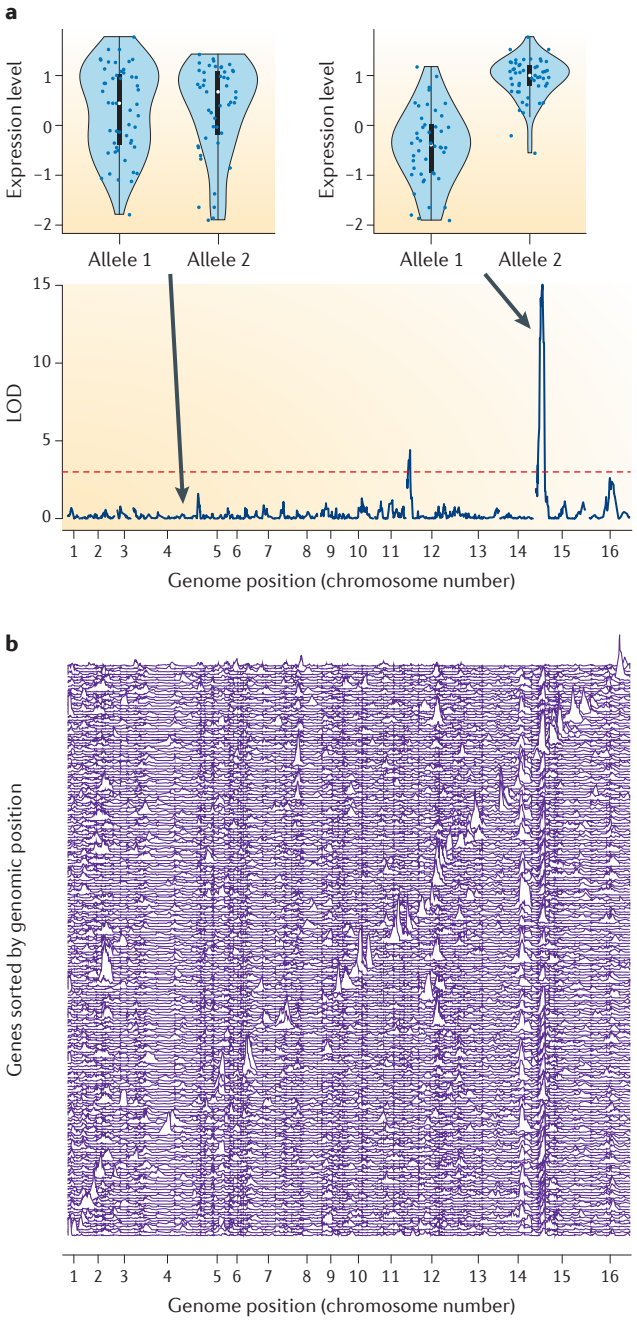


Table 1 | **A sampling of recent human eQTL data sets**

Year	Cell type or tissue studied	Design	Number of individuals	Disease or trait being compared to*	Refs
Blood					
2014	Whole blood	Twins	2,752‡	Many	63
	Whole blood	Unrelated	922	NA	47
2013	Whole blood	Unrelated	5,311§	Type 1 diabetes and cholesterol metabolism	70‖
2012	Whole blood and LCLs	Families	862	NA	216
2011	Whole blood	Unrelated	1,469	Blood traits	71
Bone					
2011	Osteoblasts	Unrelated	113	Asthma	217
Brain					
2014	10 brain regions	Unrelated	134	Parkinson's disease and other brain disorders	218
2012	Cortex and cerebellum	Unrelated	400	Parkinson's disease and other brain disorders	219
2011	Developmental time series	Unrelated	269	NA	220
2010	4 brain regions	Unrelated	150	NA	221
Heart					
2014	Heart	Unrelated	129	Cardiac traits	222
Immune system					
2014	LCLs	Unrelated	869	Type 1 diabetes and ulcerative colitis	72
	Dendritic cells	Unrelated	534¶	Autoimmune and infectious disease	74
	Lymphocytes and monocytes	Unrelated	461	Autoimmune disease and neurodegenerative diseases	73
	T cells	Unrelated	348¶	Autoimmune disease	150
	Stimulated monocytes	Unrelated	432	Immunity-related (for example, bacterial infection, inflammation, multiple sclerosis and Crohn's disease)	67
2013	LCLs	Unrelated	462	Many	88
2012	Monocytes and B cells	Unrelated	283	Immunity-related (for example, ulcerative colitis and systemic lupus erythematosus)	66
2010	Monocytes	Unrelated	1,490	Many	148
2009	Fibroblasts, LCLs and T cells	Unrelated	75	NA	8
2007	Lymphocytes	Extended families	1,240	HDL-C	49
Liver					
2011	Liver tissue	Unrelated	266	Diabetes, drug response, lipid levels and prostate cancer	223
2008	Liver tissue	Unrelated	427	Type 1 diabetes, coronary artery disease and plasma LDL-C	224
Lung					
2012	Lung tissue	Unrelated	1,111	Asthma	225
Multiple tissue types					
2012	Adipose tissue	Twins	856	Triglyceride levels and birth weight	62
	Skin tissue			Melanoma	
	LCLs			Immunity-related	
2010	Liver tissue	Unrelated	960	Plasma LDL-C and myocardial infarction	100
	Subcutaneous fat		433		
	Omental fat		520		

Organismal phenotypes
Traits that are detectable at the level of the whole organism, such as shape, size, colour, growth rate or the risk of developing a certain disease.

Linkage disequilibrium
The phenomenon whereby specific allele combinations occur more frequently than expected by chance, typically because they are physically close to each other on the same chromosome.

QTLs
Genomic regions that carry one or several DNA sequence variants which influence a continuously variable trait of interest.

Table 1 (cont.) | **A sampling of recent human eQTL data sets**

Year	Cell type or tissue studied	Design	Number of individuals	Disease or trait being compared to*	Refs
Tumours					
2014	Colorectal tumours and normal tissue	Unrelated	103	Colorectal cancer	226
	5 tumour types[#]	Unrelated	145–391	5 types of cancer	227
2013	Breast cancer	Unrelated	219	Breast cancer	228

eQTL, expression quantitative trait loci; GWAS, genome-wide association study; HDL-C, high-density lipoprotein cholesterol; LCL, immortalized lymphoblastoid cell line; LDL-C, low-density lipoprotein cholesterol; NA, none reported in eQTL paper, although overlap is often reported in follow-up analyses in the context of additional GWASs. The table shows recent studies that presented new eQTL data sets in humans. Unless otherwise indicated, meta-analyses, computational reanalyses or GWASs of diseases that compare to published eQTL data sets are not shown. The table is not meant to be exhaustive but to provide an overview of the breadth and scale of the field. *Selections of traits from those highlighted in the given paper are shown; eQTLs are usually compared to many more traits. [‡]An additional 1,895 unrelated individuals were studied in a replication data set. [§]An additional 2,775 unrelated individuals were studied in a replication data set. [‖]The largest eQTL meta-analysis so far. [¶]eQTL mapping was carried out on the basis of measurements of a targeted subset of genes. [#]Based on publicly available data.

designed experimental crosses, existing pedigrees or families[9] or unrelated individuals from natural populations — the most common eQTL study design in humans[6,11] (FIG. 1A). eQTLs are often classified according to the relative locations of the eQTLs and the gene or genes that they influence, and according to the type of mechanism through which they affect expression (FIG. 1C).

Local eQTLs. An early observation was that some eQTLs are located near the genes they influence, whereas others are located elsewhere in the genome[3]. The former have been called 'local' eQTLs[13]. Local eQTLs can influence gene expression by two different mechanisms. Most obviously, they can act in *cis* and affect expression in an allele-specific manner. By definition, each allele of such eQTLs affects only the expression of the copy of the gene that is located on the same physical chromosome with it and not the expression of the copy on the homologous chromosome. Therefore, *cis*-eQTLs can be detected in heterozygous individuals by quantifying the relative expression levels of the two alleles, for example, by counting the number of times each allele is observed in RNA sequencing data. If there is an imbalance in the expression levels of the two alleles, then the gene is affected by a *cis*-eQTL[41–46].

Local eQTLs do not always act in *cis*[13,41,45] but can also act in *trans*. *Trans*-eQTLs are due to polymorphisms that alter the structure, function or expression of a diffusible factor (FIG. 1C). The resulting differential activity or abundance of this factor alters expression levels of the genes that are influenced by the *trans*-eQTL. As the diffusible intermediate is equally available to both alleles of a target gene, *trans*-eQTLs do not lead to allele-biased expression in heterozygous individuals. Furthermore, *trans*-eQTLs can be located anywhere in the genome relative to the genes they regulate. If they happen to be close to the given gene, then they will appear as local but not *cis*-acting eQTLs. An extreme example of a *trans*-acting local eQTL is a single amino acid substitution in the yeast *AMN1* gene, which results in differential regulation of *AMN1* itself through a regulatory feedback loop that involves several additional factors[41]. Although it has become common to use the terms 'local eQTLs' and '*cis*-eQTLs' interchangeably in human eQTL studies, we advocate using the appropriate precise terminology to clearly delineate relative position from mode of action.

Local eQTLs are abundant in all species studied so far. In humans, nearly 80% of expressed genes in whole blood had a local eQTL in a recent survey of nearly 1,000 individuals[47]. In yeast, ~25% of genes had a local eQTL in a comparison of two different isolates[41], and many more local regulatory variants are expected to exist in additional yeast strains that have not been studied so far. Indeed, a population genetic extrapolation predicted that most or all yeast genes will have local eQTLs across the global yeast diversity[48].

Distant eQTLs. Distant eQTLs are defined as loci that are located further away from the genes they influence. The precise distance required for an eQTL to be distant is arbitrary and can be defined in physical or genetic distance; consequently, it differs between studies. For example, such a distance can range from 10 kb in yeast[3] to 2 Mb in humans[7]; some studies even require distant eQTLs to be located on different chromosomes from the genes they influence[49]. Distant eQTLs usually act in *trans*. The number of distant eQTLs that have been identified so far is much more variable between species than that of local eQTLs. In yeast[3,50,51], the nematode *Caenorhabditis elegans*[31], the plant *Arabidopsis thaliana*[23,25] and rodents[16,52–54], there are multiple strong *trans*-acting-eQTL hot spots that can each affect the expression of up to hundreds of genes. In yeast, many of the expression effects at some of these hot spots are caused by variation in single genes[33,50]. Other yeast hot spots may contain multiple causal genes[55,56] — a finding also seen in mouse mapping panels[16,57]. Studies of model organism panels also routinely identify large numbers of distant eQTLs that do not fall into hot spots[31,50].

In contrast to these findings in model organisms, distant eQTLs have been harder to find in human population-based samples, and family-based analyses have provided mixed support for distant-eQTL hot spots[9,58]. The apparent difference in the prevalence of distant

Homologous
Pertaining to the two copies of the same chromosome that were inherited from the mother and the father in diploid organisms (such as humans).

RNA sequencing
A method to determine the sequence of RNA molecules in a biological sample. By counting the RNA molecules that were transcribed from each gene, RNA sequencing can be used to quantify mRNA expression levels.

Genetic distance
A measure of how often two sites in a genome are separated during meiosis. Genetic distance is correlated with physical distance but can differ quantitatively because of variation in recombination rate along a chromosome.

Box 2 | **Controlling and using non-genetic sources of expression variation**

Large studies of gene expression, such as studies of expression quantitative trait loci (eQTLs), will invariably be carried out over a long time span and sometimes in multiple laboratories[108]. Even with the most standardized techniques, the resulting data sets usually contain some degree of systematic variation that is not the focus of the experiment. This can be technical (for example, batch effects due to slightly different experimental conditions) or biological (for example, different age or sex of the individuals in the study)[200]. This extra variation can obscure true signals or, worse, generate false positives if it is confounded with variables of interest[200,201].

There are many strategies to control systematic variation. When they are known (for example, sex and age, or processing date), the sources of this variation can be explicitly taken into account during analysis. Alternatively, the expression data can be used to identify principal components, surrogate variables[202] or hidden factors in a Bayesian analysis[203]. These capture large systematic effects that can then be removed before the remaining analyses, sometimes markedly improving eQTL detection in the 'de-noised' data[203]. However, strong real genetic effects (such as *trans*-acting eQTL hot spots) can inadvertently be removed by these approaches[204]. One way to avoid this is to jointly estimate confounding factors and genetic signals[205]. There are many related approaches to account for non-genetic sources of variation in eQTL mapping[206–211].

In addition to treating non-genetic variation as noise during eQTL mapping, this variation can sometimes be used to extract useful information. For instance, the gene expression data can be combined with external information (for example, on transcription factor (TF) binding motifs) to infer unobserved cellular states such as the activities of TFs[212,213] or of post-transcriptional regulators[214]. In turn, these inferred activities can be used to better understand the biological modes of action of the observed eQTL.

In another example, Francesconi and Lehner[215] reanalysed an eQTL data set in *Caenorhabditis elegans*[31]. The worms in the panel had been synchronized to the same developmental stage but still varied from each other within a range of several hours. The authors used expression data from a developmental time series to assign each strain to its precise 'developmental age' and then used this 'age' as a covariate in eQTL mapping. They found not only many more eQTLs but also QTLs that affected the dynamics of expression changes during development[215].

eQTLs between species is most likely due to the fact that the detection of distant eQTLs is more difficult in human populations than in experimental crosses. In crosses, few alleles segregate at high frequencies at each locus, and linkage blocks are relatively large, resulting in high statistical power at any position in the genome. In human population samples, there are multiple haplotypes at most positions in the genome, multiple variants per region (most of which are at low frequencies) and shorter linkage blocks. Together, these features necessitate many more association tests. The strict significance thresholds required to correct for this large number of tests result in low statistical power, making distant eQTLs harder to find. Indeed, to reduce the multiple-testing burden, most human eQTL searches have been restricted to local variation around each gene, such that distant eQTLs cannot be discovered by design. Distant eQTLs also have smaller effect sizes[59] and seem to be more tissue-specific than local eQTLs[59,60], which further complicates their detection.

Several recent studies estimated the relative importance of local and distant genetic variation on human mRNA abundance variation[61–63]. This can be achieved even without knowing the identity of individual eQTLs. Genome-wide genotyping data can be used to partition the variance in a trait into various sources such as genetic or environmental variation[64,65]. The genetic component (that is, the 'heritability') of the gene expression variance can be further partitioned into the contribution of the genomic region centred on the gene itself and the contribution of the remainder of the genome. This distant contribution accounts for the majority (60–75%) of the heritability in human gene expression[61–63]. Thus, distant eQTLs clearly exist in humans just as they do in model organisms. Indeed, as sample sizes increase in human studies, more distant eQTLs are being discovered[62,63,66–73]. In both yeast[50] and humans[67,74], the effects of distant eQTLs can change considerably in different environments. Based on the evidence so far, the *trans*-acting component in human populations seems to be more dispersed across many loci across the genome than the major hot spots seen in model organisms. It remains unclear whether this is due to the comparatively low power in human studies, a result of the fact that more variation is examined in human population studies than in crosses in model organisms, or a true reflection of biological differences.

The molecular chain of causality

The molecular nature of* cis*-eQTLs. Regulatory variation can affect organisms by interfering with any of the steps along the gene expression cascade from DNA to protein (FIG. 1B). For many years, technology largely limited eQTL studies to measures of mRNA abundance. Now, new technologies fuelled by the advances in massively parallel sequencing enable detailed examination of how sequence variation influences the individual steps of gene expression. The first wave of these studies examined transcription factor (TF) binding[75–79], chromatin accessibility[80,81], DNA methylation[82–86], alternative splicing[43,44,87–89], small RNAs[88,90,91], large intergenic non-coding RNAs (lincRNAs)[92,93], RNA editing[89] and mRNA degradation[94]. It is now clear that all of these types of transcripts and processes can be affected by regulatory variants[39]. There is emerging evidence that much of the variation at multiple genomic levels is orchestrated through *cis*-acting sequence differences that affect TF binding.

A major advance in understanding the nature of the causal DNA variants that underlie eQTLs is the growing availability of whole-genome sequences[88,95]. All sequence variants are essentially known in these studies, so that the causal eQTL variants themselves (rather than a linked single-nucleotide polymorphism (SNP) on a genotyping array) will often show the highest association with gene expression. For example, a recent eQTL mapping of 462 fully sequenced human individuals found that short insertions and deletions (indels) are more likely to result in local eQTLs than SNPs[88]. Causal eQTL variants are further enriched in DNase I-hypersensitive sites, in regions annotated[96] as active promoters and strong enhancers, and in TF binding sites[88].

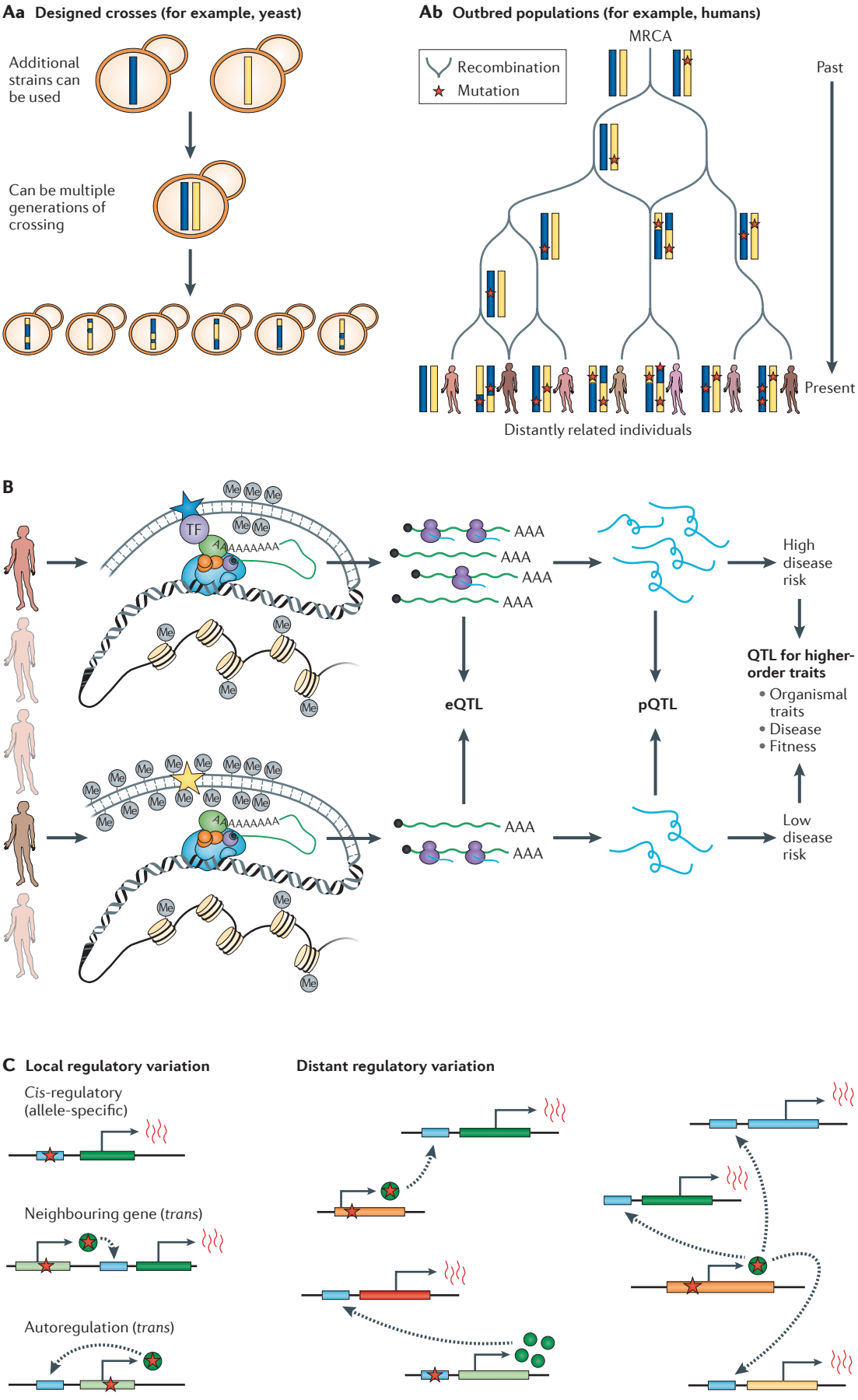
Investigation of allele-specific histone modifications, TF binding and mRNA levels in human parent–offspring trios has provided additional support for the importance of variation in TF binding[97–99]. Sequence variants that are located in TF binding sites are correlated not only with variation in TF binding itself but also with

Linkage blocks
Continuous haplotypes that are not broken up in the population under study such that sequence variants within them all show the same patterns of association with a certain trait of interest.

Haplotypes
Stretches of DNA that carry certain combinations of alleles at two or more DNA variants.

Variance
A statistical measure of the variability of a trait in a population.

Heritability
The fraction of variance in a trait that is due to genetic differences among the individuals in a population.



◀ Figure 1 | **Designs for genetic mapping of variation in gene expression and other molecular traits.** Molecular variation is mapped in genetically variable populations. **Aa** | These populations can be generated through designed crosses in model organisms. For example, the genetic backgrounds of a set of yeast strains are reshuffled by mating followed by meiosis, resulting in a set of recombinant offspring. **Ab** | Alternatively, outbred populations can be used that carry genetic variation which was spread and recombined by historical genetic processes (illustrated by the genetic history of a hypothetical region of the genome). This is the most popular design for expression quantitative trait locus (eQTL) mapping in humans. Pedigrees or families of related individuals can also be used (not shown). **B** | The molecular quantity of interest is measured in each individual in the study panel. The figure illustrates the results for two individuals that differ in the expression of a certain gene. To map the loci involved (marked by the star), this molecular variation is compared to genetic variation among the individuals (BOX 1). Many of the steps along the gene expression cascade can be studied in this way, including DNA methylation (Me), histone modifications, transcription factor (TF) binding, active transcription, mRNA levels (resulting in eQTLs), translation and protein levels (resulting in protein QTLs (pQTLs)). In the example, the altered protein level due to the genetic polymorphism influences disease risk, an organismal trait. **C** | eQTLs can be classified according to their location (local or distant to the gene they influence) and according to their mode of action (*cis* or *trans*). MRCA, most recent common ancestor.

differential histone modifications, mRNA levels and DNA methylation[84]. A parsimonious explanation for these observations is that differential TF binding is the primary molecular change. In turn, the TFs then direct the changes in histone modifications, DNA methylation and mRNA expression.

These global analyses complement multiple examples of individual human *cis*-eQTLs that are caused by sequence differences in TF binding sites[49,74,100–103]. However, only 25–35% of genetically variable TF binding events are associated with a known sequence variant within the corresponding TF binding core motif[77,79,98,99]. Some of the remaining cases may be due to missed motif variants. Alternatively, causal sequence variants outside core motifs may influence TF binding, perhaps by altering the local shape of the DNA or by influencing the binding of other TFs that form complexes with the assayed TF. An interesting variation on this theme is a 2-bp deletion in a promoter that segregates among yeast strains and causes variation in the expression of the *ERG28* gene[104]. The deletion allele does not disrupt a TF binding motif but instead moves two neighbouring TF binding sites closer together, resulting in reduced binding by both factors. Experimental dissection of additional *cis*-eQTLs will reveal the full spectrum of their molecular causes.

The molecular nature of* trans*-eQTLs. *Trans*-eQTLs can be due to a diverse set of molecular causes. They can be coding variants in regulatory genes or local eQTLs of such genes. Work in yeast showed that the regulatory genes that act as *trans* factors themselves have diverse functions. Proteins encoded by *trans*-acting yeast eQTLs are not enriched for TFs[33]. Instead, the functions they encode range from RNA-binding proteins (such as *MKT1*)[55,105,106] to members of signalling cascades (such as *IRA2* (REF. 50) and *GPA1* (REF. 33)) and modifiers of nucleosome composition (such as *swc5* in *Schizosaccharomyces pombe*[51]).

Recent work in humans is beginning to reveal similar molecular diversity among *trans*-eQTLs[66–68,70,74]. For example, *IRF7* (which encodes interferon regulatory factor 7, a transcription factor) is influenced by a local eQTL in activated dendritic cells, a type of immune cell. The same eQTL SNP is associated with the expression of a set of genes in *trans*[74]. Experimental overexpression of *IRF7* influences the same set of genes, demonstrating that the altered expression of *IRF7* caused by the local eQTL is responsible for driving further expression changes[74]. Adding another level of complexity, in human monocytes *IRF7* is influenced by another *trans*-acting locus that maps to a local eQTL in *EBI2* (also known as *GPR183*, which encodes a G protein-coupled receptor)[69]. Other *trans*-eQTLs in humans include an amino acid substitution in a cytochrome P450 enzyme[67] that reduces the half-life of the protein[107], a local eQTL for the *LYZ* gene encoding the secreted enzyme lysozyme[66–68], and multiple associations within the highly variable human major histocompatibility complex (MHC) region[66,67]. When more human *trans*-eQTLs have been fine-mapped, it will be interesting to examine whether certain types of molecular causes (coding versus regulatory) or genes (encoding TFs, signalling molecules or others) are more likely to result in *trans*-acting variation.

From the transcriptome to the proteome. The vast majority of current studies use mRNA rather than protein abundance as the measure of gene expression. However, coding genes ultimately function through their protein products. The experimental preference for mRNA is because transcript levels can be measured more easily than protein levels. Whereas eQTL studies can rely on standardized methods that readily quantify most of the transcripts in the genome[108], the few studies that have examined proteome variation used a wide range of assays, including commercial antibody kits for certain blood proteins[109], 2D difference gel electrophoresis[110], microwestern arrays[111] and different types of mass spectrometry[112–118]. Many of these studies were limited in the number of samples and/or proteins.

Two questions arise when comparing genetic influences on the transcriptome and the proteome. Does a typical eQTL feed forward into variation in protein levels? Conversely, are most pQTLs simply a reflection of the underlying mRNA variation, or do they arise from genetic influences on post-transcriptional mechanisms? These questions are an important area of active research, and a consistent picture has yet to emerge. The first direct comparisons of eQTLs and pQTLs obtained by mass spectrometry in model organisms[57,119,120] suggested that most eQTLs did not seem to influence protein levels and, conversely, that most pQTLs seemed to arise without corresponding differences in mRNA abundance.

More recent proteome-wide studies reported better agreement between eQTLs and pQTLs. For example, an analysis of 22 diverse yeast strains found that most of the identified eQTLs had concordant effects on protein levels[118]. Studies in human cell lines have not only reported substantial overlap between eQTLs and pQTLs but also detected a number of protein-specific pQTLs without

apparent effects on mRNA[111,112,117]. The effects of eQTLs seemed to be attenuated at the protein level relative to their effects on mRNA levels[112]. These studies used modest numbers of individuals (<100) so that only local loci with large effects could be examined. Some of the 'missing' eQTLs or pQTLs might therefore have been due to low statistical power.

To overcome the limitation of sample size, a novel experimental design was recently introduced in yeast. The approach allows the detection of distant and local pQTLs by comparing the genetic make-up of pools of single cells with very high protein levels to those with very low protein levels from populations of hundreds of thousands of genetically different cells[121,122]. Both studies showed that more than half of the known distant eQTLs that previously seemed to be mRNA-specific do in fact have concordant pQTLs. Importantly, the high detection power also revealed that a typical protein is affected by several times more distant pQTLs than seen before. It remains to be seen whether the many newly discovered pQTLs only arise at the protein level or whether they reflect eQTLs with small effects that have not been detected so far.

Protein translation may provide a plausible target for sequence variants that influence protein, but not mRNA, levels[123,124]. However, recent studies that used ribosome profiling[125] in yeast[126,127] and in humans[112] found strong concordance between eQTLs and ribosome occupancy QTLs, suggesting that protein-specific genetic effects are likely to act beyond the stage of translation.

Lessons from model organisms

Testing causality of human variants. Model organisms can provide insights into the consequences of sequence variants that are suspected to be important in human disease. For instance, it is possible to engineer the genome and observe the effects of a given sequence variant on the whole organism rather than on a molecular or cellular trait. A regulatory element upstream of the *MYC* oncogene (which encodes a TF), for example, contains a SNP that is associated with cancer in humans. The SNP alleles lead to differential expression of *MYC*[128], but the association between differential expression of *MYC* and cancer was inconclusive. To test this, transgenic mice were generated in which the mouse orthologue of the SNP-containing *cis*-regulatory element (CRE) was deleted[129]. Ablation of the CRE resulted in modestly reduced *Myc* expression levels. Crucially, the CRE-deleted mice were also more resistant to tumorigenesis. However, although this work shows that the CRE is an important determinant of cancer risk, it does not prove that the SNP within the element contributes to this risk through gene expression. To formally achieve this, it would be necessary to generate transgenic mice that differ in the SNP alleles.

The *Myc* study in mice was facilitated by the fact that the orthologous CRE actually exists in mice, in spite of the fact that individual regulatory elements turn over rapidly in mammalian evolution[130]. Many other human CREs would have to be inserted into the mouse genome in order to study their sequence variation. For example, transgenic mice were created that carried a human CRE that is not present in mice and that regulates the KIT ligand (*Kitl*) gene[131]. The mice were engineered to carry either of the alleles of a human SNP inside the CRE. The SNP was suspected to contribute to blond hair in humans. The two CRE alleles drove differential expression of *Kitl* in the skin and also resulted in a difference in mouse coat colour, suggesting that this variant may influence human physical appearance through altered gene regulation.

Genetic associations shared between species. The powerful mapping panels in model organisms are designed to maximize statistical power to detect genetic associations from sets of defined genetic backgrounds. The panels can be used to identify novel links between sequence variants, expression and disease[40]. Although the individual causal DNA variants are most probably not the same as those in humans, the same genes, pathways and networks may nevertheless harbour important genetic variation in several species. For example, eQTL maps from a panel of genetically heterogeneous rats revealed a network of immune-related genes[69] centred on the TF-encoding gene *Irf7*. In turn, the expression of *Irf7* is influenced in *trans* by a *cis*-acting eQTL for the *Ebi2* gene. Remarkably, an expression network with a similar structure to that in rats was also found in human monocytes[69]. As in rats, the human network was also influenced in *trans* by a *cis*-eQTL for *EBI2*. One of the human *EBI2 cis*-eQTL SNPs was found to be associated with the autoimmune disease type I diabetes, an association that had been missed previously.

Conceptual insights into causality. In addition to applications with direct relevance for human disease, model organisms can provide powerful conceptual insights into the relationship between regulatory variation and organismal traits. Genetic tools that allow precise genome engineering and experimental control of gene expression make yeast an especially suitable organism for such studies. These advantages allowed the first identification of a *trans*-acting eQTL that causes trait variation through expression changes. The protein encoded by the *AMN1* gene differs between a common laboratory budding yeast strain and other strains at two amino acids[33]. The Amn1 protein is a negative regulator of *ACE2*, which encodes a TF[132]. In turn, Ace2 activates *CTS1* (REF. 133), which encodes chitinase, an enzyme required for cell separation[134]. The laboratory strain allele of Amn1 fails to repress *ACE2*, resulting in upregulation of *CTS1* and separation between mother and daughter cells. In other strains, *CTS1* expression is lower, and budding cells stick together to result in 'clumpy' growth[33].

Recent work in yeast has provided further insights into how genetic effects on expression shape phenotypes (FIG. 2). Rest *et al.*[135] showed that effects on fitness of the expression level of the essential gene *LCB2* are nonlinear and depend on both the environment and the genetic background (FIG. 2A). They replaced the native *LCB2* promoter with a promoter system that can be used to finely control *LCB2* expression and found that the

reduction in expression level compared with the wild-type level led to a sharp decrease in fitness. By contrast, overexpression had only minor fitness effects. Growth in the presence of osmotic stress preserved the shape of the expression–fitness curve but shifted it such that higher expression of *LCB2* was required for a given fitness level. Wild yeast strains had lower expression levels of *LCB2* than those seen in the laboratory strain, suggesting that the expression–fitness curve is also shifted by different genetic backgrounds[135].

Yeast eQTL hot spots often overlap with loci that influence growth rates. This observation could arise when gene expression differences cause growth differences or vice versa, as well as when expression and growth do not affect each other but are both caused by the same locus or by different loci in close proximity (FIG. 2C). To distinguish among these scenarios, Gagneur *et al.*[136] mapped yeast eQTLs in five environmental conditions and compared the eQTLs to loci that influence growth in the respective conditions. Whenever a QTL affected growth in a given condition, this locus also affected the expression of multiple genes in that condition; that is, the locus was an eQTL hot spot. However, some QTLs affected growth in only a few conditions but remained eQTL hot spots even in conditions in which they did not affect growth. When a growth QTL was detected in several conditions, it was also an eQTL for some genes in all of these conditions but for other genes in only some of the conditions. The authors used a statistical model to show that those genes influenced by a hot spot irrespective of growth condition were more likely to cause growth differences than genes with condition-dependent eQTLs[136].

Two studies used allelic engineering to show that four nucleotide changes in three TFs together explain nearly all of the variation in sporulation efficiency between two yeast isolates[137,138] (FIG. 2B). By contrast, these same four variants accounted for much less variation in gene expression at the time point at which cells switch to the meiotic state[137]. The effects of these SNPs on gene expression had different magnitudes and different degrees of additive versus epistatic contributions compared with those on sporulation, suggesting that there is no simple direct relationship between the effects of these specific nucleotide variants on gene expression and on the cellular phenotype. Together, these yeast studies show that the relationship between gene expression variation and higher-order traits can be highly complex, and they provide context for interpreting links between eQTLs and disease in humans.

The role of eQTLs in human disease

A region identified by GWASs as associated with disease typically contains more than one gene and multiple sequence variants that are in linkage disequilibrium with each other. The next task is to find the causal genes and sequence variants, and to understand how they affect the disease. Although variants that alter coding sequences are obvious candidates, most human GWAS hits fall far from coding regions of genes and are over-represented in regulatory elements[34,139]. Therefore, most causal variants probably influence traits by altering gene expression. A GWAS hit typically contains multiple regulatory elements, and these elements can influence genes at some distance[140]. It is therefore not easy to pinpoint the causal variants and to discern the genes that they affect. eQTLs can provide the crucial link between the variants in a GWAS region and the biological processes they affect.

Bigger data, better maps. When a GWAS hit is also an eQTL for a given gene, this provides the hypothesis that the expression of this gene influences the disease. It is now firmly established that GWAS hits for common diseases are enriched for eQTLs, and vice versa. This enrichment was first noted in a comparison of associations for traits such as body mass index to eQTLs in adipose tissues and blood[10], and was later observed in comparisons of GWAS results and eQTLs mapped in immortalized cell lines[141,142]. There have since been many eQTL studies in tissues with relevance for a given disease, providing numerous cases of GWAS–eQTL pairs that suggest plausible causal mechanisms (TABLE 1).

To maximize the chance of finding GWAS–eQTL links, eQTLs need to be mapped in additional tissues[143,144]. For example, Kapoor *et al.*[145] identified a causal regulatory variant that influences heart function by altering an enhancer in heart tissue; this variant had been missed in previous eQTL studies of different tissues. Reference panels of eQTLs identified in multiple human tissues[146] will be useful in this regard, as are current efforts to map eQTLs in purified primary individual cell types (such as certain subtypes of immune cells[66–68,73,74,147–151]) rather than in tissues that contain a mixture of cell types (such as whole blood). Such analyses of purified cell types are especially important because eQTL architectures can change dynamically during developmental differentiation of related cell types, as demonstrated in the haematopoietic cell lineage in mice[152,153]. For tissues that are difficult to obtain from primary sources, induced pluripotent stem cells (iPSCs)[154], which can be differentiated into various cell types, provide a promising alternative. As a crucial prerequisite for using iPSCs, it was recently shown that gene expression differences between iPSCs derived from several donors are larger than the technical variation induced by cellular reprogramming and cell culture[155].

Even in tissues and cell types that have been studied, the currently available maps are incomplete. This is partly because eQTL architectures can change considerably in cells exposed to different growth conditions, as initially demonstrated in yeast[50,106,136]. A rapidly growing set of studies is mapping eQTLs in human cells exposed to physiologically relevant stimuli. So far, the main focus of this work is on comparing eQTLs in unstimulated immune cells[66,68,73,147–149,151] to eQTLs that are only seen after these cells have been activated by triggering the immune response[16,67,74,150,156,157]. Immune cell activation can reveal large numbers of eQTLs that are hidden in resting cells[158]. Stimulus-dependent eQTLs in whole blood also help to explain individual differences in the transcriptional response to vaccination[159].

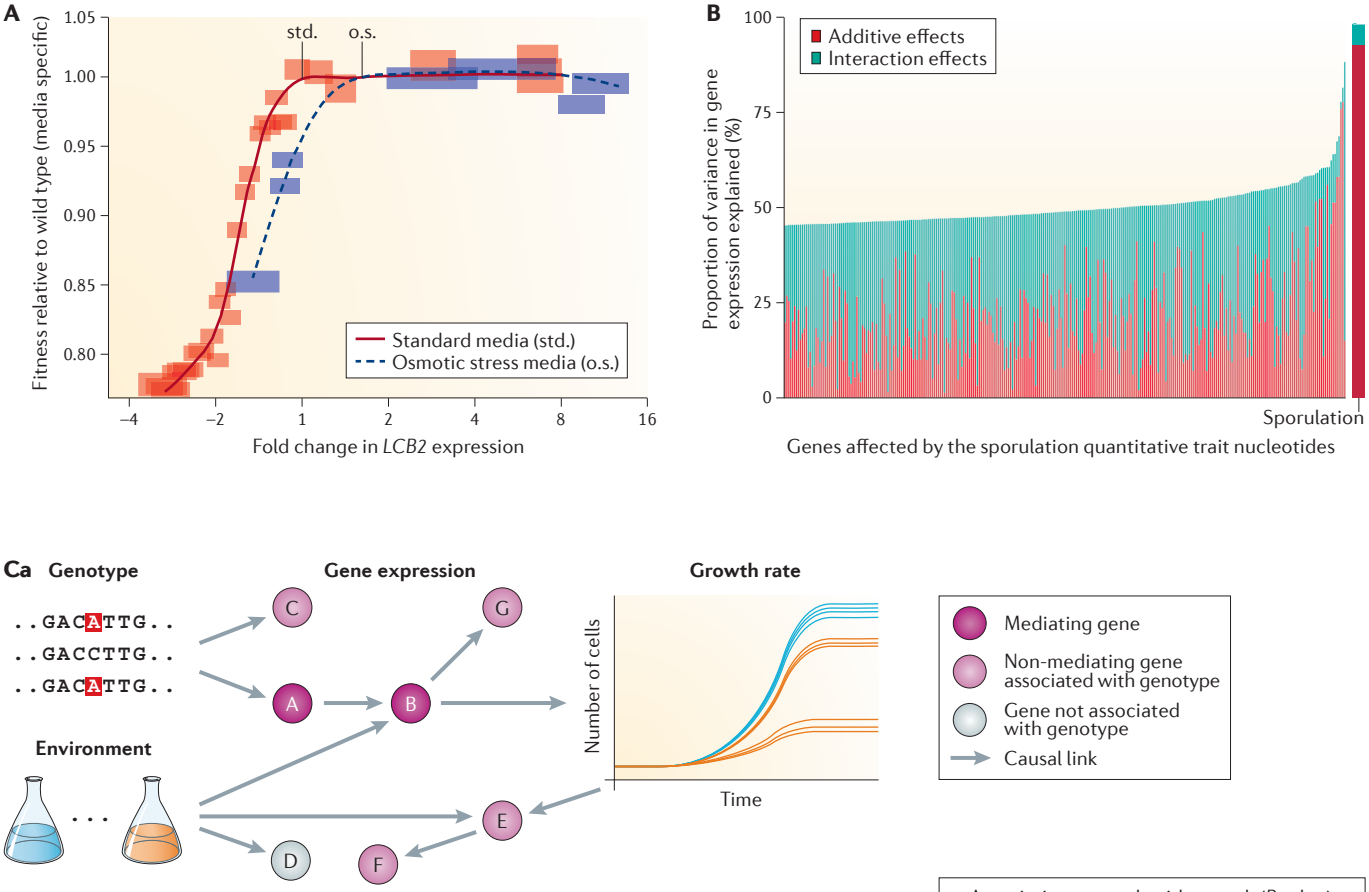


Figure 2 | **Key insights into the causal relationship between eQTLs and organismal traits provided by recent studies in yeast.** **A** | Fitness consequences of experimentally altered expression of the *LCB2* gene are shown[135]. Note that even low levels of upregulation or downregulation can have drastically different consequences on fitness, depending on the baseline expression level and the environment. **B** | Genetic effects on gene expression caused by four well-characterized single-nucleotide polymorphisms (SNPs) that had been originally identified through their effects on yeast sporulation efficiency[137]. The genes differ from each other in the degree to which their expression is influenced by the four SNPs and in the relative importance of epistatic effects compared with additive effects. For comparison, the effects of the four SNPs on sporulation efficiency are shown in the bar furthest to the right. The impact of these SNPs on the organismal trait (sporulation) cannot be simply explained by their effect on the expression of any one gene. **C** | Gagneur *et al.*[136] studied the possible flows of causality from DNA variation between two yeast strains (Y and S) to gene expression and yeast growth rate (part **Ca**). The correspondence of expression quantitative trait locus (eQTL) hot spots (black peaks) and growth QTLs (coloured bars) varies substantially among different environments (part **Cb**). Part **A** reproduced from Rest, J. S. *et al.* Nonlinear fitness consequences of variation in expression level of a eukaryotic gene. *Mol. Biol. Evol.* (2013) **30**(2), 448–456, by permission of Oxford University Press. Parts **B** and **C** from REF. 137 and REF. 136, respectively.

Many eQTLs are missed owing to low statistical power in small samples. As sample sizes increase towards thousands of individuals, eQTL catalogues have grown remarkably. Recent studies of a thousand or more individuals report eQTLs for the majority of genes expressed in a given tissue[47,63,70]. Many of these eQTLs have small effects that were beyond the detection limit of the earlier, smaller panels, and there are likely to be thousands more eQTLs beyond our current statistical reach.

Analytical challenges and opportunities. A common approach for prioritizing likely causal variants among the variants that are linked to a genomic region implicated by GWASs is to focus on variants that are eQTLs in a published data set (TABLE 1) and on variants that are located in functional elements such as promoters or enhancers[160–163]. Similar to eQTLs, maps of such regulatory features are available in a growing number of tissues and cell types[96,161]. As both eQTL catalogues and maps of regulatory features continue to grow, more and more eQTLs will be found to colocalize with regulatory regions, requiring decisions on which overlaps are the most informative. Furthermore, GWAS signals would ideally be compared to eQTLs and regulatory features obtained from the 'causal' cell type in which the given disease emerges. However, for many diseases and traits it is not a priori clear which cell type is the most relevant.

Recently, integrative Bayesian methods have been developed to identify sets of functional sequence annotations (for example, from sets of regulatory elements from many different cell lines) that are the most relevant for the given trait in an unbiased manner[139,164–168]. These methods complement similar approaches to fine-map the causal sites within eQTLs themselves[47,105,169,170], to jointly analyse eQTLs across different tissues[171] and to predict expression levels from genotypes[172]. When annotations inferred to be important for the trait come from a certain tissue, this suggests that the tissue is biologically important for the trait. Not only can these links support known connections (for example, between high-density lipoprotein (HDL) levels and regulatory elements in liver cells[164]), but they can also generate new hypotheses. For example, GWAS hits for platelet volume were enriched in regulatory elements in the spleen, which is not obviously connected to this trait[164]. In turn, the annotations inferred to be the most important can then be used to select the most promising variants for future study.

The approaches show promise in fine-mapping individual GWAS regions[165] but often still result in sets of multiple potentially causal variants. A natural extension of these methods is to also consider whether each variant is an eQTL[166,168]. For example, in one analysis eQTLs were found to be the most important source of information when prioritizing among GWAS hits for autoimmune disorders[166]. Further information can be gained by including published functional knowledge about the gene that the eQTL regulates and by studying whether it is known to have functions related to the disease[173]. This type of information will be particularly useful when a given eQTL influences the expression of multiple genes, perhaps in different tissues. Other Bayesian approaches formally test whether an eQTL and a GWAS hit are due to the same causal variant rather than to two closely linked variants[174], and may gain predictive power by considering functional annotations. As the diverse genomic data sets continue to grow, rigorous integration across them will be crucial.

Bridges across the causality gap. Several examples of causal links between human regulatory variants and disease have been uncovered in recent years[49,101–103,145,175,176] (see REF. 177 for a review of earlier work). A prominent example is a common SNP at 1p13, a locus associated with the risk of myocardial infarction[100] (FIG. 3). Remarkably, although the SNP is located in the 3′ untranslated region of a gene, its causal effect on infarction is not mediated through this gene. Instead, the minor SNP allele, which is associated with reduced risk, creates a binding site for a TF that is preferentially expressed in the liver[100]. As a consequence, the sortilin 1 (*SORT1*) gene, which is ~40 kb away and separated from the causal SNP by 2 additional genes, is upregulated specifically in the liver. Knockdown and overexpression studies in mouse liver confirmed that higher expression of the sortilin protein results in lower levels of low-density lipoprotein cholesterol (LDL-C). In turn, LDL-C is a well-known risk factor for myocardial infarction, providing the final link in the causal connection between this eQTL and a major human disease.

A second case involves SNPs associated with obesity that are located within introns of the fat mass and obesity-associated (*FTO*) gene[178]. As knockout of *Fto* results in leaner mice[179], it had been suspected (but not demonstrated) that the causal variants might act through expression levels of *FTO*. However, recent work showed that the genomic region containing the variants in the obesity-associated region is in physical contact with the iroquois homeobox 3 (*IRX3*) gene, which is located at a distance of ~500 kb from the variants[180]. SNPs in the obesity-associated region also showed association with the expression of *IRX3* in human cerebellum, albeit with only nominal significance. Phenotypes of mouse knockout models of *Irx3* are consistent with a causal role of *IRX3* in obesity. More work will be needed to identify the precise causal variant or variants within the obesity-associated region and to determine whether their effects on obesity are mediated primarily by *IRX3* expression variation.

Notably, these examples both involve SNPs in regulatory sequences that are located some distance away from the genes they influence. Such long-range regulatory interactions are usually mediated by physical contact between the regulatory DNA and the regulated gene. Targeted[181–185] and global[186] methods for mapping the physical interactions of GWAS hits will be useful to systematically dissect the regulatory consequences of disease-associated variants, as recently demonstrated for several breast cancer risk loci[184].

Conclusions and perspectives

The work we have reviewed here demonstrates that eQTLs have important roles in influencing downstream traits ranging from yeast growth and fitness to human disease. The connections along the causal chain from DNA variant to altered expression and trait variation can be surprisingly complex. Dissection of the functional impact of regulatory variation will continue to require careful experimental follow-up work. The existing and rapidly growing catalogues of eQTLs will enable more precise targeting of these efforts. Large sample sizes will be crucial to ensure that no relevant eQTLs are missed, especially the more elusive *trans*-acting variants. To ensure that the relevant biology is captured, eQTL maps need to be constructed in a wide range of tissues and cell types, and under a variety of physiologically important conditions. When tissues are difficult to obtain or when they represent complex mixtures of cells (such as brain

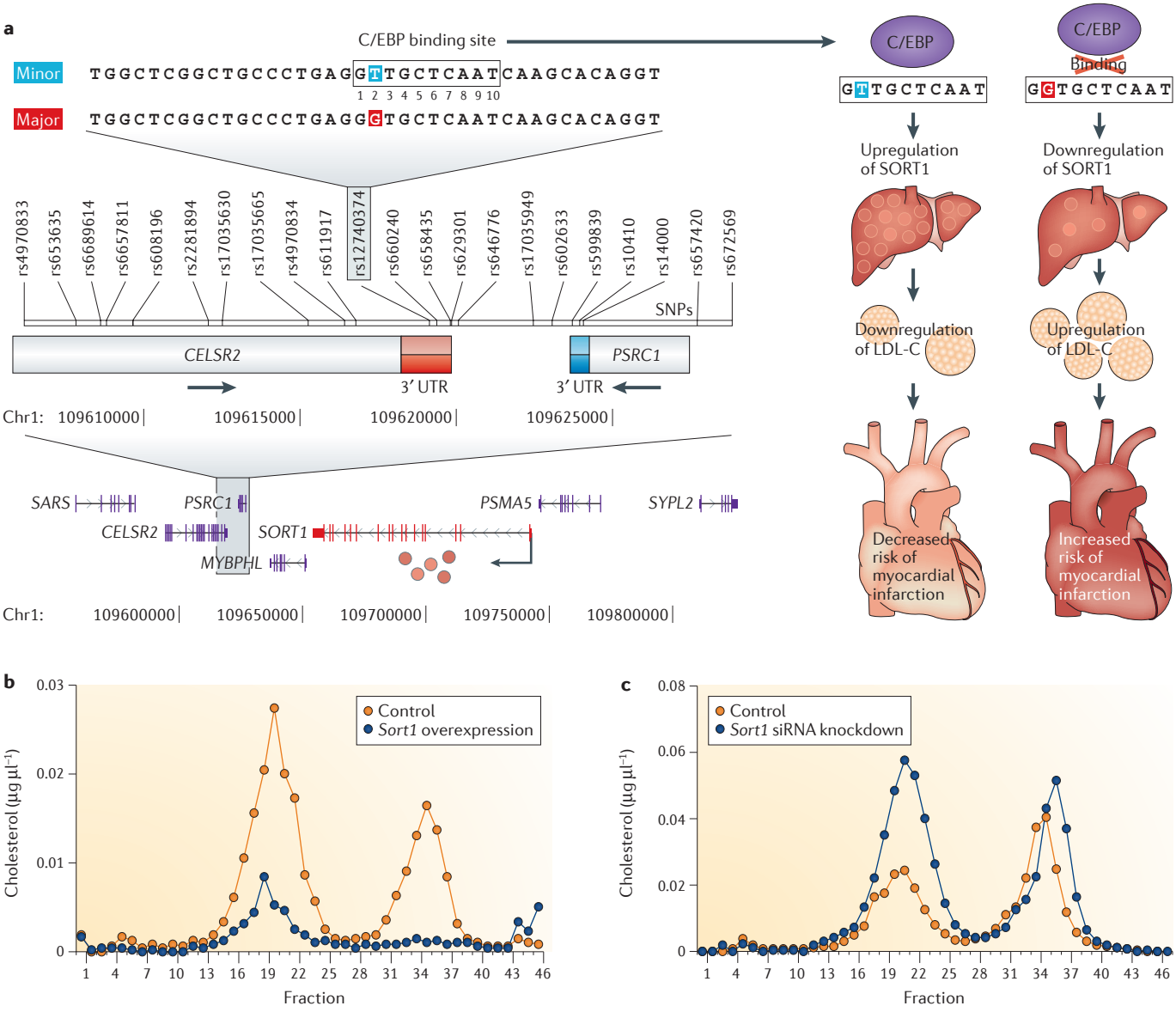


Figure 3 | **An example of a full chain of causality in humans.** **a** | The minor allele of a non-coding single-nucleotide polymorphism (SNP) in the 3′ untranslated region (3′UTR) of the *CELSR2* (cadherin, EGF LAG seven-pass G-type receptor 2) gene creates a transcription factor binding site for CCAAT/enhancer-binding protein (C/EBP), to which the major allele does not bind[100]. Binding of C/EBP at this site leads to increased expression of the sortilin 1 (*SORT1*) gene in liver cells. **b** | In mice, overexpression of *Sort1* in the liver reduces low-density lipoprotein cholesterol (LDL-C) levels (calculated using fractions 10–26). **c** | Small interfering RNA (siRNA)-mediated knockdown of *Sort1* increases LDL-C levels in mice. As LDL-C, in turn, is a known risk factor for myocardial infarction, this work provides a complete causal path from a non-coding variant to altered risk for a major human disease. *SORT1* is separated from the causal SNP by two additional genes, and the causal effect on LDL-C is not mediated through *CELSR2*, although the causal SNP is in the 3′UTR of this gene. Chr1, chromosome 1; *MYBPHL*, myosin-binding protein H-like; *PSMA5*, proteasome (prosome, macropain) subunit, alpha type, 5; *PSRC1*, proline/serine-rich coiled-coil 1; *SARS*, seryl-tRNA synthetase; *SYPL2*, synaptophysin-like 2. Figure adapted from REF. 100, Nature Publishing Group.

tissues), differentiated iPSCs[187] provide promising alternatives. Methods to study expression patterns in single cells obtained directly from dissociated complex tissues[188,189] might also be useful in this regard. Prioritizing putative causal links between DNA variation, expression and phenotypes will require further development of sophisticated data integration techniques.

Furthermore, in addition to studying the downstream effects of eQTLs, it will be equally important to more completely explore the genetic and molecular architecture of gene expression variation. Are expression levels themselves as polygenic as many disease traits, or do they have simpler architectures? How varied are the genetic architectures of the thousands of genes in a genome[190]? Does the importance of epistatic variance differ between gene expression[56,191–194] and higher-order traits[195,196]? How plastic are gene expression architectures in different environments[50,67,74,106,192,197]? A major challenge is the identification of large catalogues of causal variants that underlie eQTLs. Rapid advances in genome editing — for example, using the CRISPR–Cas9 (clustered regularly interspaced short palindromic repeat–CRISPR-associated protein 9) system[198,199] — will greatly speed up our ability to experimentally validate and investigate the effects of putative causative variants. Ultimately, a complete genetic and molecular understanding of how genetic variation shapes gene expression and cell biology will be useful for improving predictive models of the consequences of new mutations and for personalized genomic medicine.

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Acknowledgements
The authors thank members of L.K.'s laboratory and many colleagues for discussions. L.K. is supported by funds from the Howard Hughes Medical Institute, the US National Institutes of Health and the James S. McDonnell Foundation. F.W.A. was supported by the German Science Foundation (DFG).

Competing interests statement
The authors declare no competing interests.